Original Research Article

Molecular docking studies on phenolic constituents of *Anethum graveolens* seed extracts

ABSTRACT

Background: There are studies indicating that aqueous or hydroalcoholic dill extracts showed higher antioxidant activity compared to other fractions. Molecular docking studies would be relevant to get the mechanism of acting the phenolic constituents of *Anethum graveolens* seed extracts as bioactive compounds.
Methodology: In order to perform the docking studies of antioxidant activity of phenolic constituents of *Anethum graveolens* seed extracts, BIOVIA Discovery Studio and AutoDock Vina softwares were used.
Results: It was proved that orientation of the flavonoids within Hck and CYP2C9 binding sites is the major reason which determine their inhibitory potency.
Conclusion: Molecular docking studies indicate that the compounds identified interact with the target enzymes Hck and CYP2C9 at molecular level through their condensed ring systems and hydroxyl substituents and therefore support the antioxidant capacity of the studied phenolic compounds.

Keywords: Dill seeds, extracts, reference ligands, molecular targets

1. INTRODUCTION

Clinical studies have shown that very common chronic age-related diseases have increased oxidative stress. The ability of phytochemicals to lower the level of free radicals is associated with their benefits in the prevention of chronic diseases caused by oxidative stress and it may be associated with their antioxidant activity [1,2]. Natural polyphenols, such as phenolic acids, flavonoids and coumarins, are the most important group of secondary metabolites found in many medicinal plants as well as in the human diet and contribute to the antioxidant properties of plants and foods [1,3].

Synthetic phenolic antioxidants are scarcely accepted due to their ineffectiveness and toxicity [4, 5]. As the risk factors for the development of lethal diseases among population are constantly increasing, there is a global tendency to use natural substances from medicinal herbs and food plants as therapeutic antioxidants [4, 5].

Plants used as aromatic herbs or spices are often underestimated by their lack of metabolic profile, as they are often important sources of bioactive compounds [6]. There are two

related species of cultivated dill, the European dill (*A. graveolens*) which is widely distributed throughout the world and the Indian dill (*A. sowa*) cultivated throughout the Indian sub-continent, Malaysia and Japan [7,8]. Since the Egyptian period, dill has been used as a condiment and also for medicinal purposes as an ingredient in pain relievers [7,8]. *A. graveolens* is used in India in the preparation of more than 56 ayurvedic medicines, which are usually prescribed in abdominal discomfort, colic and digestive disorders [8], while in Ethiopia dill is one of the plants used in the traditional treatment of kidney diseases [3]. In Romania, the whole dill plant is used in the traditional cuisine to flavor different foods, and the seeds are used as spices for pickles. Hydroalcoholic and hydroglycerin extracts are recommended for emmenagogue, estrogenic, galactogogue, and female hormone regulator, anti-inflammatory, antispasmodic, diuretic, stomachic and carminative effects. Dill seed extract showed significant protective gastric activities, having anti-ulcer effects in mice by oral administration of hydrochloric acid and absolute ethanol [9], antinociceptive properties in inflammatory pain [10], antioxidant activities [11], anticonvulsant effect against pentylenetetrazole [12], antimicrobial activity against bacteria, such as *Bacillus subtilis*, *Escherichia coli*, *Helicobacter pylori*, *Salmonella sp*., *Shigella flexneri, Staphylococcus aureus, Pseudomonas aeruginosa* or fungi such as *Aspergillus flavus, Candida albicans, Fusarium graminearum* [7,8,13].

Dill seeds contain up to 5% volatile oil – rich in carvone and limonene as major compounds, flavonoids, coumarins, xanthones and terpenes [3,8]. Several studies have been done to determine the chemical composition of the essential oil of dill seeds and it has been shown to exert antioxidant activity, suggesting a possible application as a natural preservative for food, as well as a protector of the body against oxidative stress [13,14]. However, essential oils are volatile and difficult to store due to low stability, while extracts are easier to prepare, cheaper and more stable. There are studies indicating that aqueous or hydroalcoholic dill extracts showed higher antioxidant activity compared to other fractions [15,16]. To the best of our knowledge, there are few reports concerning the chemical composition of the alcoholic and hydroalcoholic extracts of *Anethum graveolens* seeds and the data differ from author to author, as different components have been analyzed [5,17,12,18]. Dill seeds are marketed in Romania, but it is not yet known how detailed the plant product has been studied from a chemical point of view, so its value as food/nutraceutical. Moreover, dill seeds have many medicinal properties. Based on these considerations, molecular docking studies were conducted to understand the mechanism of antioxidant activity of dill seed extracts.

2. MOLECULAR DOCKING METHODOLOGY

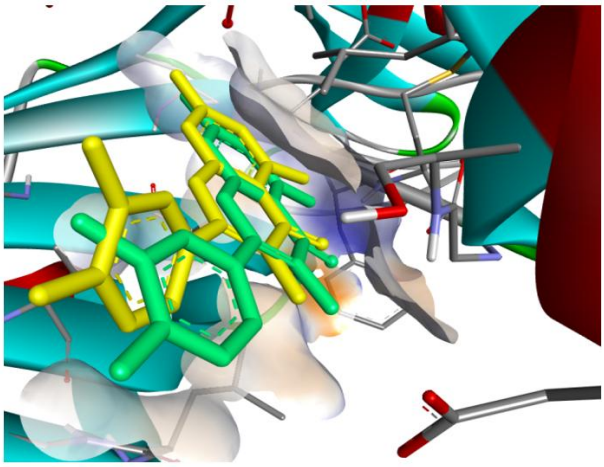
The crystal structures of Hck (2HCK) and CYP2C9 (1OG5) were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) [19,20] as PDB files and used after removal of all bound water molecules, ligands and cofactors by using BIOVIA Discovery Studio software [21] (BIOVIA, San Diego, CA, USA). Polar hydrogen atoms and Gasteiger charges were added using AutoDock Vina software (Scripps Research, San Diego, CA, USA). All other settings were left as default [22]. The ligands were obtained from the PubChem database in SDF (structure-data file) format and were converted to the PDB format using https://cactus.nci.nih.gov/translate/. The ligands were prepared by adding Gasteiger chargers, removing non-polar hydrogens and defining all rotatable bonds. Docking was performed by placing the ligands in the binding pocket of the protein (together in one PDB file) and the binding affinity was calculated with AutoDock Vina software. The coordinates of the grid center for 2HCK are 31.47x, 45.34y and 98.79z, grid size 24x, 24y, 24z while the coordinates for 1OG5 are -20.256x, 86.991y and 38.581z, grid size 24x, 24y, 24z. BIOVIA Discovery Studio software6 (BIOVIA, San Diego, CA, USA) was used to design the 2D diagrams and to analyze the data obtained.

3. RESULTS AND DISCUSSION

Molecular docking is a commonly used method to predict the preferred orientation and binding energy of a small molecule (ligand) to a target macromolecule (receptor). This can be useful for understanding the mechanism of action and the nature of ligand binding to different types of receptors [23]. Molecular docking studies were performed to evaluate the antioxidant potential of phenolic compounds identified in the extracts A-H. The aim of these studies was to establish the relationship between in vitro results and in silico experiments. Two proteins were selected as targets for antioxidant compounds, tyrosine kinase Hck [24] and cytochrome P450, CYP2C9 [25,26].

Protein kinases are enzymes commonly found in eukaryotes that are involved in various cellular processes such as cell cycle, proliferation, metabolism and apoptosis. Activation of protein-tyrosine kinases can induce controlled production of endogenous hydrogen peroxide (H_2O_2) [27]. In contrast, excessive generation of H_2O_2 and other reactive oxygen species (ROS) can alter some enzymes, including protein-tyrosine kinases. The generation of abnormal protein-tyrosine kinases and exacerbation of oxidative stress can lead to damage of biomolecules and pathologies such as cancer, diabetes, neurodegenerative and cardiovascular diseases. Therefore, it can be anticipated that inhibitors that block tyrosine kinases activity and the signaling pathways they activate could alleviate clinical symptoms and provide a useful basis for the development of new drugs [27,28]. Cytochrome P-450 proteins (CYP450) are ubiquitous hemoxygenases that play a critical role in the oxidative biotransformation of a wide range of physiologically important substances as well as exogenous compounds such as drugs, environmental compounds and pollutants [25,26]. CYP2C9 is the predominant isoform of CYP450 with a major contribution to human drug metabolism [26]. CYP450 is also known to generate ROS during arachidonic acid metabolism [29].

Therefore, we performed an in silico evaluation of the binding poses and functional group interactions between the phenolic derivatives identified in *A. graveolens* extracts and the catalytic sites of Hck and CYP2C9 in order to examine the potential molecular interactions between these compounds and the target enzymes. The molecular docking was first conducted on compounds known as reference ligands and the results were compared with these known molecules used as positive controls. Quercetin was chosen as reference ligand for Hck (quercetin-Hck complex, PDB code: 2HCK) [24], while S-warfarin was chosen as the reference ligand for the protein CYP2C9 (S warfarin - CYP2C9 complex, PDB code: 1OG5) [25]. To validate the method, a ligand model was built and docked into the crystal structure of the target enzyme. The docked ligand model was superimposed on the reference model of ligand taken from the protein data bank. Root mean square deviation (RMSD) between the co-crystallized ligand and the docked ligand showed a value of 1.097 Å for 2HCK (Figure 1) and 0.89 Å for 1OG5 (Figure 2), respectively, which fits within the generally accepted values of 1.5-2 Å [29].



= co-crystallized = re-docked

Fig. 1. Data obtained in the validation of molecular docking methods for Hck target enzyme. Quercetin in 2HCK complex, Quercetin binding affinity of - 9.4 kcal/mol, RMSD = 1.097 Å

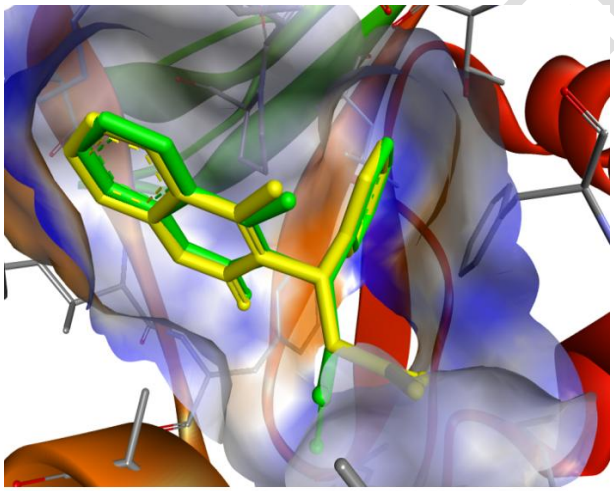


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Fig. 2. Data obtained in the validation of molecular docking methods for CYP2C9 target enzyme. S-warfarin in 1OG5 complex, S-warfarin binding affinity of - 9.9 kcal/mol, RMSD = 0.890 Å.

The identified phenolic compounds (Figure 3) were docked within the active site of the target macromolecules and the binding affinity of the different conformations of the inhibitors was calculated with AutoDock Vina software. As can be seen in Table 1, the best docking scores

were obtained by compounds with condensed ring systems: quercetin, kaempferol and (±) - catechin belonging to the flavonoid family, followed by the coumarin umbelliferone.

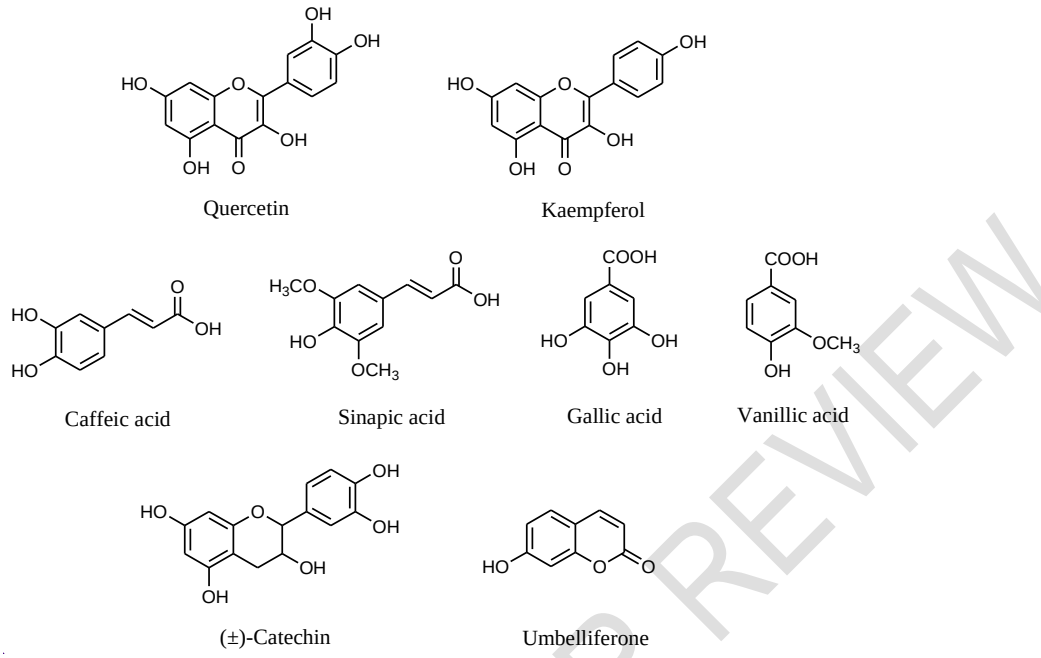

Fig. 3. Phenolic compounds screened in *A. graveolens* seed extracts.

The binding interactions of the docked conformations of ligands with target proteins are identified in 2D diagrams obtained using BIOVIA Discovery Studio software6 (BIOVIA, San Diego, CA, USA). The amino acids at the active site of the target protein as well as the functional groups through which the studied compounds bind to these amino acids can be seen in these diagrams.

Table 1. Binding affinity within 2HCK and 1OG5 of the phenolic compounds identified in *A. graveolens* extracts

Compound	Binding affinity (kcal/mol)	
	2HCK	1OG5
Quercetin	-9.4	-8.7
Kaempferol	-9.0	-8.6
Caffeic acid	-6.4	-6.1
Sinapic acid	-6.8	-6.2
Gallic acid	-5.8	-5.9
Vanillic acid	-6.0	-5.5
(±)-Catechin	-8.7	-8.1
Umbelliferone	-7.1	-6.3
S-warfarin	-	-9.9

The study of 2HCK complex shows that the active site of Hck is coated by LEU273, GLY274, ALA275, VAL281, ALA293, VAL323, THR338, GLU339, PHE340, MET341, GLY344, SER345, ALA390, LEU393 and ALA403 residues. Specific interactions occur between these residues and the condensed rings of quercetin: hydrogen bonds (GLU339, MET341), Pi-sigma interactions (LEU393, VAL281), Pi-alkyl interactions (ALA293, LEU273, VAL281). In

addition, the phenyl ring in position 2 is involved in hydrogen bonds (ALA390), as well as Pi-sigma interactions (VAL281). Several van der Waals interactions occur between the quercetin molecule and the enzyme residues (PHE340, SER345, GLY274, ALA275, ALA403, THR338, VAL323), as well as one carbon-hydrogen bond (GLY344). Due to its similar structure, kaempferol showed a similar binding affinity to the protein (- 9.0 kcal/mol). Comparing the 2D diagrams of these two compounds, it can be seen that the protein binding mode is very similar, suggesting that these flavonoids may inhibit Hck activity with similar potency. The small difference in binding affinity may be caused by the lack of one hydroxyl group on the phenyl ring of kaempferol resulting in the lack of the hydrogen bond with ALA390. Catechin interacted with seven residues common to quercetin (GLU339, THR338, LEU273, ALA403, LEU393, VAL281, GLY344), that are involved in hydrogen bonds, Pi-sigma and Pi-alkyl interactions and therefore revealing a high affinity of this compound for Hck (- 8.7 kcal/mol) (Figure 4). The binding affinities of the compounds and the control molecule are similar due to the related chemical structures. However, the lower binding affinity of catechin may suggest that the orientation of flavonoids within the catalytic site may influence their inhibitory capacity.

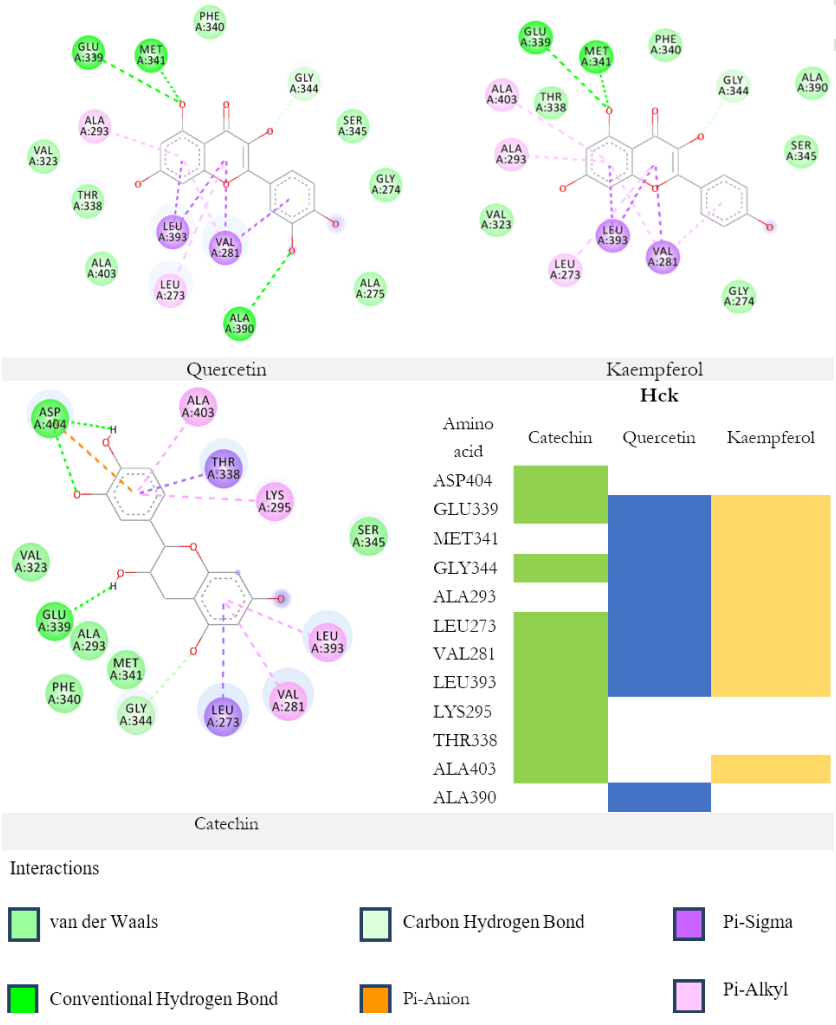

Fig. 4. Interactions of the flavonoids identified in *A. graveolens* extracts with Hck enzyme.

Several studies have reported that flavonoids are effective inhibitors of protein kinases [30,31,32] and through this action flavonoids can regulate processes such as cell proliferation, immune response, inflammation or blood clotting, preventing pathologies such as cancer, diabetes, neurodegenerative and cardiovascular diseases [31]. From the 2D diagrams of flavonoid-Hck complexes it can be concluded that the condensed ring systems and their hydroxyl substitutions are important structural features for Hck binding.

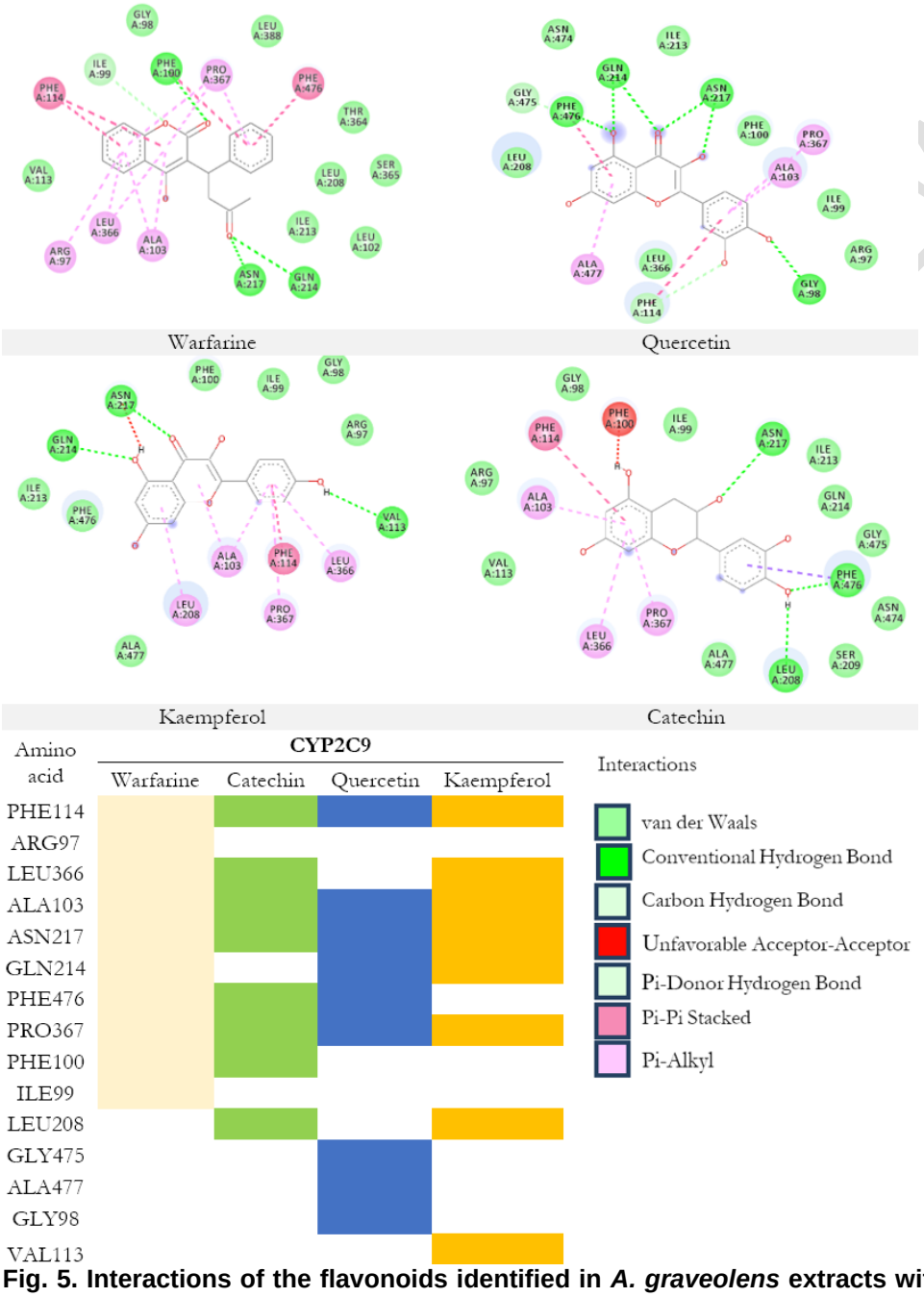

Fig. 5. Interactions of the flavonoids identified in *A. graveolens* extracts with CYP2C9 enzym.

The study of the 1OG5 complex shows that the active site of CYP2C9 is surrounded by ARG97, GLY98, ILE99, PHE100, LEU102, ALA103, VAL113, PHE114, LEU208, ILE213, GLN214, ASN217, THR364, SER365, LEU366, PRO367, LEU388 and PHE476 residues which interact with the control ligand S-warfarin through hydrogen bonds (PHE100, ASN217, GLN214), Pi-Pi (PHE114, PHE476) and Pi-alkyl interactions (PRO367, ARG97, LEU366, ALA103), one carbon - hydrogen bond (ILE99) and van der Waals interactions (GLY98, LEU388, THR364, LEU208, SER365, ILE213, LEU102, VAL113) which is in agreement with published data [25]. Quercetin docked in a different orientation to that of warfarin, with the condensed rings positioned adjacent to PHE476, GLN214, ASN217, ALA477 and LEU208 residues, three of which being involved in hydrogen bonds (PHE476, GLN214 and ASN217). From the list of residues, six of them interact with quercetin (ALA103, PHE114, GLN214, ASN217, PRO367 and PHE476) which indicates a good antioxidant ability of this compound (binding affinity value - 8.7 kcal/mol). Kaempferol docked in an orientation similar to quercetin, making hydrogen bonds with GLN214, ASN217 and VAL113 residues, suggesting that these flavonoids may inhibit CYP2C9 activity with similar potency (binding affinity value - 8.6 kcal/mol), while catechin docked in a more warfarin-like orientation, forming bonds with common residues in CYP2C9 binding site. Although catechin docked with a similar number of hydrogen bonds (ASN217, PHE476 and LEU208) as warfarin, the binding affinity of catechin (- 8.1 kcal/mol) is lower to that of warfarin (- 9.9 kcal/mol). The unfavorable binding to PHE100 could be a reason. In addition, although the hydroxyl group on the non-planar catechin ring is involved in hydrogen bonds, it is probably unfavorable from an energy point of view (Figure 5).

These results demonstrate that hydrogen bonds and hydrophobic interactions occur between ligands and target proteins, stabilizing the ligands at the catalytic site and providing high binding affinities. The unique stability of the ligands in the binding sites can be attributed to the large number of Pi-interactions such as Pi-sigma, Pi-anion, Pi-Pi and Pi-alkyl. It also appears that orientation of the flavonoids within Hck and CYP2C9 binding sites, rather than the number of hydrogen bonds formed, may determine their inhibitory potency.

4. CONCLUSIONS

Molecular docking studies provide information about possible molecular targets and support the antioxidant capacity of the studied phenolic compounds. Subsequent chemical analysis will provide a valuable contribution to the knowledge of the phytochemical composition of this edible plant.

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